

Special Issue of Photodynamic Therapy and Photodetection with Porphyrin Precursors for the *Journal of Environmental Pathology, Toxicology, and Oncology*

Alcira Batlle

Guest Editor

Centro de Investigaciones sobre Porfirinas y Porfirias (CIPYP), Argentine National Research Council (CONICET) and University of Buenos Aires (UBA). Viamonte 1881
10A 1056 Buenos Aires, Argentina
batlle@mail.retina.ar

Qian Peng

Editor

PDT Lab. Department of Pathology, The Rikshospitalet-Radiumhospitalet HF, University of Oslo, Montebello, 0310 Oslo, Norway
qian.peng@labmed.uio.no

Preface

The collection of articles put together in this special issue originated from a selection of papers presented at the 5th International Workshop on Photodynamic Therapy and Photodetection with Porphyrin Precursors, held in Buenos Aires, Argentina, June 22–24, 2006. This meeting was attended by leading scientists from 13 countries of the world, whom we have had the pleasure and honor of having been closely associated with or known for a long time. The four previous International Workshops on Photodynamic Therapy with Aminolevulinic Acid had been organized by Professor Stanley Brown, at Leeds in the United Kingdom, in the periods of April 29–31, 1995; April 24–26, 1998; April 14–16, 2000; and April 19–21, 2002.

Porphyrin synthesis is one of the most fundamental attributes of all living cells. Porphyrins are considered “the pigments of life” as coined by Lemberg and Ledge in 1944. Porphyrins are the only photosensitizers synthesized in the cells, and the best examples of these endogenous sensitizers are the porphyrin intermediates formed and accumulated in the cutaneous porphyrias, producing the characteristic skin photosensitization.

Photodynamic therapy (PDT) is a promising new modality of cancer treatment, which involves the combination of a photosensitizing agent that is taken up selectively and retained by tumoral cells, and light of an appropriate wavelength. Separately, each of these factors is harmless by itself, although when combined in the presence of oxygen, cytotoxic reactive oxygen species are produced, leading to irreversible cellular damage and causing cell death and tumor destruction.

After either exogenous administration or endogenous synthesis, porphyrins finally accumulate in higher proliferative cells. Light energy absorbed by the photosensitizer (PS) can produce fluorescence. The tumor-localizing properties of the PS have been extensively employed for photodetection (PD) and diagnosis, as well as for the PDT, of tumors.

Light was used thousands of years ago for treating vitiligo by the Chinese, Indians, and Egyptians; however, the actual birth of PDT was estab-

lished roughly 100 years ago by the work of Raab and von Tappeiner in Munich. But the current interest in PDT only resurfaced in the early 1970s with the use of the water-soluble derivative of Hematoporphyrin (HPD). Most of the clinical PDT work has been carried out with this HPD or Photofrin; however, this PS was yet far from ideal. That was the reason why other PSs have been developed; among these is the powerful PS protoporphyrin IX (PPIX). Thus, endogenous synthesis of PPIX from its natural precursor, aminolevulinic acid (ALA), for PDT (ALA-PDT) and PD (ALA-PD) has been extensively investigated during the last two decades.

The earliest report on porphyrin biosynthesis from ALA in a highly dividing plant tissue, considered as a vegetable tumor, came from Batle’s laboratories in 1975. In this soybean callus tissue system, addition of ALA to the culture medium was found to stimulate porphyrin accumulation, prevent growth, and lead to tissue death. Some 12–15 years later, these same authors and others reported on the selective destruction of different highly dividing cells by photoactivation of endogenous PPIX formed from ALA.

Higher synthesis and accumulation of PPIX in rapidly proliferating cells provides the biological rationale for the clinical use of ALA-PDT and ALA-PD. Multiple efforts have been and are currently being put forward in order to optimize the efficacy of the process, including the development of different PSs (in particular, a number of more hydrophobic ALA derivatives) and testing of different methods of drug delivery, drug selectivity, and light delivery, and combinations of therapies.

In the session on Porphyrin Synthesis and Delivery Systems of this meeting, Drs. Alison Curnow and Andrew Pye, in their paper, *Biochemical Manipulation via Iron Chelation to Enhance Porphyrin Production from Porphyrin Precursors*, reported on their in vitro studies investigating the efficacy of the new iron chelator, 1,2-diethyl-3-hydroxypyridin-4-one hydrochloride (CP94), and the known iron chelator, desferrioxamine (DFO) at enhancing PPIX fluorescence in cultured human lung fibroblasts and squamous carcinoma cells with ALA and ALA-methyl ester (MAL). Interestingly, CP94 increased PPIX fluorescence when given ALA/MAL; compared with

ALA/MAL alone, CP94 was superior to DFO, accumulating the same levels of PPIX within a shorter time period. In conclusion, clinical application of CP94 to increase ALA/MAL-PDT could result in greater accumulation of PPIX or could be employed to reduce the time of the drug-light interval.

Dr. Ryan Donnelly and his group, in their paper, *Formulation and Characterization of Poly(ethylene glycol)-based, 5-aminolevulinic Acid-loaded Solid Dosage Forms Intended for Photodynamic and Photodiagnostic Methodologies in the Colorectal Region*, have shown that ALA-loaded poly(ethylene glycol) (PEG) disks prepared using three MW PEG matrices, were of potential for rectal administration as part of PDT and PD colorectal procedures. Low concentrations of ALA (1% w/w) were completely dispersed throughout the PEG matrix, with PEG 1000 being the most suitable for melting at about body temperature. The conclusion was that the simple formulation designed containing 1% ALA in a PEG 1000 matrix can be applied directly to the colorectal area, and it is a good alternative to per oral ALA dosing.

Another paper on this session came from the same Irish group of Desmond Morrow, Martin Garland, Paul McCarron, David Woolfson, and Ryan Donnelly, namely, *Innovative Drug Delivery Strategies for Topical Photodynamic Therapy Using Porphyrin Precursors*. Although clinical use of ALA and its effects within target cells are subjects of intensive research, studies on design and evolution of effective delivery systems for PS application have been less investigated. In this paper, the authors present an updated review on the conventional approaches to topical delivery of ALA and its esters, and highlight a number of recent innovative strategies developed to enhance the efficacy of ALA-PDT. Among them, most interesting are the physical techniques using needle-free jet injectors and microfabricated microneedle arrays, which have the great potential to ease the delivery of relative high-MW PSs across the skin, allowing their penetration to deep lesions and instant drug delivery.

In the session on Mechanisms of Action of PDT with Porphyrin Precursors, Herbert Schneck-enburger and his group in the paper, *Cell-substrate Topology on ALA-PDT Using Variable-angle Total Internal Reflection Fluorescence Microscopy (VA-TIRFM)*,

described their studies on the fluorescence of PPIX in human cancer cells after application of ALA prior to and after irradiation with sublethal light doses, using a specific illumination device for total internal reflection fluorescence microscopy (TIRFM) and a highly sensitive electron multiplying (EM) CCD camera. Cell-substrate distances were generally decreased on light exposure, while focal contacts were maintained, showing that light-induced detachment of cells from their substrate was not occurring.

The paper, *Fluorescence Monitoring of a Topically Applied Liposomal Temoporfin Formulation and Photodynamic Therapy of Nonpigmented Skin Malignancies*, by Prof. Katarine Svanberg and her colleagues at the session on PD and PDT of Skin and GI Lesions with Porphyrin Precursors, describes the study, for the first time, of a liposomal meso-tetra (hydroxyphenyl) chlorine (mTHPC)(INN:Temoporfin) gel formulation for topical application, in relation with PDT of nonpigmented skin malignancies in humans. The intervals between Temoporfin administration and light irradiation were of four hours. They have investigated the PS distribution within the tumor and the surrounding normal skin before, during, and after PDT, finding high tumor selectivity. There was no pain, nor swollen tissue, nor red- dening, as is often the case for ALA-PDT.

At the session on PD and PDT of Bladder Lesions with Porphyrin Precursors, Prof. Bart Grossman presented a paper, *Improving the Management of Bladder Cancer with Fluorescence Cystoscopy*. His studies have shown that fluorescence cystoscopy can reveal carcinoma in situ, which could be missed under conventional white-light cystoscopy. Fluorescence cystoscopy also increased detection of papillary tumors. Results with ALA have demonstrated that resection of bladder cancer with fluorescence leads to improved disease-free survival when compared with resection under white light.

Dr. Sigrid Kvaal and Dr. Trond Warloe, in their paper, *Photodynamic Treatment of Oral Lesions*, at the session on PD and PDT of Otorhinolaryngologic and Ovarian Lesions with Porphyrin Precursors, have found that systemic administration of ALA was better than local application of ALA in the treatment of precancerous lesions such as oral leukoplakia. PDT, after topical administration of me-

thylene blue and methyl-ALA, showed improvement in the diseases of oral mucous membrane such as oral lichen planus. They have also tested the bactericidal action of PDT on oral plaque. The authors proposed that PDT might also play a role in dental disorders instead of mechanical cleaning or antibiotic treatment.

At the session on PD and PDT of Brain Tumors with Porphyrin Precursors, Steen Madsen, Khishigzaya Kharkhuu, and Henry Hirschberg presented a paper, *Utility of the FP98 Rat Glioma Model for Photodynamic Therapy*. They have investigated the feasibility of a synergistic rat brain tumor model of F98 glioma cells in Fischer rats, for its use in PDT studies. They have demonstrated that in vitro, the F98 cell line was sensitive to ALA-PDT at low light irradiances. Histologically, the F98 tumors shared many of the fundamental characteristics of human GBMs, such as rapid growth and infiltration. High light fluences delivered at low power caused significant edema after ALA-PDT in normal brain, so care should be taken to choose the light irradiation parameters when treating tumor-bearing animals. Rats inoculated with F98 cells preincubated with ALA showed a significant survival after light exposure. In conclusion, they have demonstrated that their F98 rat glioma model is good for PDT investigation of malignant gliomas.

Then, Herbert Stepp and coworkers, in their paper, *ALA and Malignant Glioma: Fluorescence-guided Resection and Photodynamic Treatment*, reported on clinical studies aimed to investigate fluorescence-guided resection (FGR) and PDT after oral administration of ALA in malignant glioma tissue. It has

been a phase III trial comprising an ALA group and a conventional white-light group, performed in 18 clinics in Germany. They have found that mean PPIX fluorescence in tumors was more than 100-fold increased, when compared to normal cortex. FGR contrast-enhancing tumor was completely resected in 65% of patients in the ALA group, while it was 36% in the white-light group, and progression-free survival was also superior in the ALA group.

Finally, the paper of the Argentinian team, *5-aminolevulinic Acid-mediated Photodynamic Therapy on Hep-2 and MCF-7c3 Cells*, reported on the cytotoxic effect of ALA-induced PPIX on two human carcinoma cell lines, namely, MCF-7c3 cells and Hep-2 cells. They have shown that PPIX content depended on ALA concentration and incubation time for both cell lines and treatment of the cells with desferrioxamine, previous to ALA exposure, enhanced the levels of PPIX. After incubation with ALA for five hours and radiation, 24 hours post-PDT, revealed, for the MCF-7c3 cells, typical morphological changes of necrosis, while the Hep-2 cells showed characteristic apoptosis. In the MCF-7c3 cells, PPIX was accumulated in the perinuclear region, while instead in the Hep-2 cells, it was localized in lysosomes. The authors conclude that the different mechanisms of cell death observed in both cell lines were dependent on the different intracellular localization of PPIX.

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Alcira Batlle and Qian Peng